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THE DUAL MECHANISM OF IRON ABSORPTION IN BEAN ROOT AND LEAF TISSUES

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ABSTRACT

The possible existence of a dual pattern of Fe uptake in root and leaf tissues of bean plants was investigated employing a modification of the tea-bag technique. Experimental solutions, consisting of $FeCl_3$ in 0,5 mM $CaCl_2$ solution, varied from 0,01–0,5 mM in the "low" range and from 1–30 mM in the "high" range. Absorbed Fe was determined by means of atomic absorption spectroscopy and radiometric analysis.

The Fe uptake curves for both root and leaf tissues displayed a hyperbolic rise followed by a plateau in the low concentration range, and an exponential increase in the high concentration range. The possibility exists that Fe in the high concentration range is precipitated in the tissues.

UITTREKSEL

DIE TWEEFASE MEGANISME VAN YSTEROPNAME IN WORTEL- EN BLAARWEEFSEL VAN DIE BOONTJIEPLANT

Die moontlikheid dat Fe-opname in wortel- en blaarweefsel van die boontjieplant volgens 'n tweefase patroon geskied, is ondersoek deur van 'n wysiging van die teesakkie-tegniek gebruik te maak. Eksperimentele oplossings bestaande uit $FeCl_3$ in 'n 0,5 mM $CaCl_2$-oplossing het van 0,01–0,5 mM in die "lae" gebied en van 1–30 mM in die "hoë" gebied gevarieer. Opgeneemde Fe is deur middel van atoomabsorpsie-spektroskopie en radiometriese analise bepaal.

Die krommes vir Fe-opname deur sowel wortel- as blaarweefsel het 'n hiperboliese styging gevolg deur 'n plato-gebied in die lae konsentrasiereeks, en 'n eksponensiële toename in die hoë konsentrasiereeks vertoon. Die moontlikheid bestaan dat Fe in die weefsel mag neerslaan in die hoë konsentrasiegebied.

INTRODUCTION

Epstein and others found that the rate of ion uptake over a wide concentration range follows two distinct patterns differing in magnitude (Epstein, 1966; Torii and Laties, 1966). On account hereof it has been suggested that there are at least two mechanisms of ion uptake—one operating at an external ion concentration up to 1 mM (mechanism 1) and the other from 1 mM upwards (mechanism 2).

Such a two-phase pattern of uptake has been obtained with a number of cations and anions. Epstein (1976) lists several experiments in which the biphasic absorption pattern was obtained. Out of a total of 61 experiments only six were conducted with divalent cations, of which one was with Fe. In that particular investigation it was found that Fe uptake by excised rice roots displayed a duality in uptake (Kannan, 1971).

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On account of the above findings it was decided to investigate the possible existence of a dual pattern of Fe uptake in root and leaf tissues of bean plants.

MATERIAL AND METHODS

Seeds of kidney bean (*Phaseolus vulgaris* L. cv. Top Crop) were germinated in quartz sand moistened with deionised water. After 10 days the seedlings were transferred to containers filled with full-strength culture solution—solution 1 of Hoagland and Arnon (1938), but with Fe-EDTA as Fe source—and allowed to grow for a further 10 days in a growth chamber—daily photoperiod 14 h (28 ± 2 °C) and light intensity of c. 9000 lux.

At this time, discs 5 mm in diameter were cut from the two youngest fully-expanded leaves, and the young root tips (10–15 mm) were excised. Both leaf discs and root tips were immediately transferred to deionised water. Each sample consisted of 50 leaf discs and an equal fresh mass (c. 0,3 g) of root tips.

The tissue samples were then transferred to nylon gauze bags divided into separate compartments—an adaptation of the tea-bag technique of Epstein, Rains and Elzam (1963)—in order to ensure that both root and leaf tissues were subjected to strictly comparable conditions. The bags were thereupon placed in an aerated intermediate solution of 0,5 mM $CaCl_2$, kept at 30 °C for one h, and subsequently transferred to the experimental solution, from which the uptake of Fe was studied. The latter was also continuously aerated and kept at 30 °C in a water bath in diffuse light.

Experimental solution

In preliminary trials, experimental solutions at pH 5,3 were used. However, since extremely variable and inexplicable results were obtained (e.g., absorption decreased, rather than increased with time), and Fe tended to precipitate above pH 3,2, subsequent experimental solutions were adjusted to pH 3,2 with dilute HCl. At least 150 mℓ of experimental solution per bag (containing 0,3 g each of leaf discs and root tips) were used.

The concentration of Fe (as $FeCl_3$, in 0,5 mM $CaCl_2$ solution) varied from 0,01 to 0,5 mM (''low'' range), and from 1–30 mM (''high'' range). In one series of investigations ^{59}Fe was added as a tracer to the experimental solutions (2 μCi ^{59}Fe per litre, as $FeCl_3$, obtained from the Radiochemical Centre, Amersham). In the second series of experiments, the actual amount of Fe in the tissues was determined by means of atomic absorption spectroscopy.

In all experiments the absorption period was 20 minutes; in the second series this was followed by desorption in a 10 mM Na_2-EDTA solution for 20 minutes at 2 °C.

Analysis of plant material

Following the absorption periods in the different experimental solutions, each sample was rinsed for a total of one minute in a separate series of four beakers each containing 200 mℓ deionised water. Where a desorption treatment was employed, the samples were then transferred to the desorption solution, following which they were rinsed again as above. Where ^{59}Fe had been used, radioactivity of the tissues was determined by means of a well scintillation counter. Corrections were made for radioactive decay and the total amount of Fe was calculated. Where the Fe content of the samples was determined by means of atomic absorption spectroscopy, the method of Allen (1970) was employed.

Statistics

Two replicates of each treatment were employed; each experiment was repeated on two consecutive days. The mean and standard error for each treatment were calculated. Differences between means of more than twice the standard error were regarded as significant.

RESULTS AND DISCUSSION

In Figures 1 and 2 the pattern of Fe uptake are shown with and without a desorption treatment respectively. The similarity in the shape of the curves is very striking. According to Torii and Laties (1966), the uptake of chloride in the range of 0–0,5 mM is hyperbolic; at high concentrations (1–50 mM) a second, multiple hyperbolic isotherm is obtained in the case of vacuolated tissues, whereas the isotherm for non-vacuolated tissue rises exponentially. According to these authors

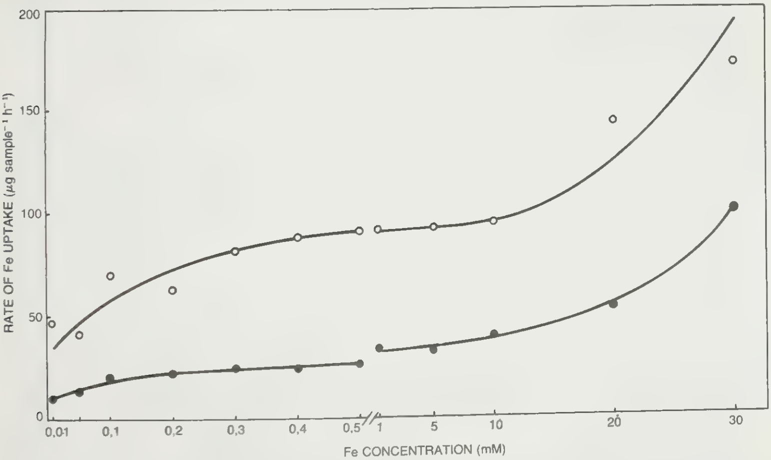

FIG. 1.

Rate of Fe absorption by bean leaf discs (●—●) and excised root tips (○—○) at low (0,01–0,5 mM) and high (1–30 mM) concentration ranges of Fe. Fe supplied as $FeCl_3$ in 0,5 mM $CaCl_2$ solution. Absorption period 20 minutes at 30 °C, followed by desorption in 10 mM Na_2-EDTA at 2 °C for 20 minutes.

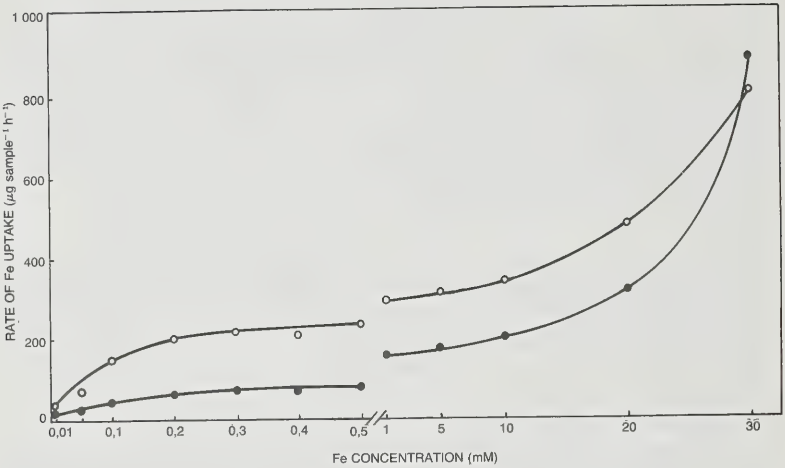

FIG. 2.

Rate of Fe absorption by bean leaf discs (●—●) and excised root tips (○—○) at low (0,01–0,5 mM) and high (1–30 mM) concentration ranges of Fe. Fe supplied as $FeCl_3$ labelled with ^{59}Fe in 0,5 mM $CaCl_2$ solution. Absorption period 20 minutes at 30 °C.

a linear to exponential increase is an indication of uptake by diffusion. It must be assumed that the tissues in this investigation (leaf discs and root tips) were vacuolated, as were the rice roots in the investigations of Kannan (1971).

As mentioned earlier, Kannan (1971) claims that Fe uptake by excised rice roots displays the dual pattern of uptake. According to his findings the absorption isotherm in the low concentration range is hyperbolic, whereas it is almost linear in the high range. He further applies the Lineweaver-Burk kinetic analysis to his data in both the low and high concentration ranges, whereas according to Epstein (1972) this should not be possible in the case of the high concentration range (mechanism 2).

A Lineweaver-Burk kinetic analysis of the present results yielded a straight-line relationship in the low concentration range for both root tips and leaf discs (Fig. 3). However, this relationship was not obtained in the high concentration range. This is in contrast to the findings of Kannan (1971) with excised rice roots.

If a dual pattern of uptake is considered as a first followed by a second hyperbolic isotherm as originally suggested by Epstein (1966), the conclusion can be drawn that Fe uptake by root and leaf tissues of the bean plant displays no true dual pattern. If, however, a curve displaying a hyperbolic rise and a plateau area, followed by either a hyperbolic, linear, or even an exponential increase, is still considered as indicative of a two-phase uptake pattern, the uptake of Fe in the present investigation can also be regarded as biphasic.

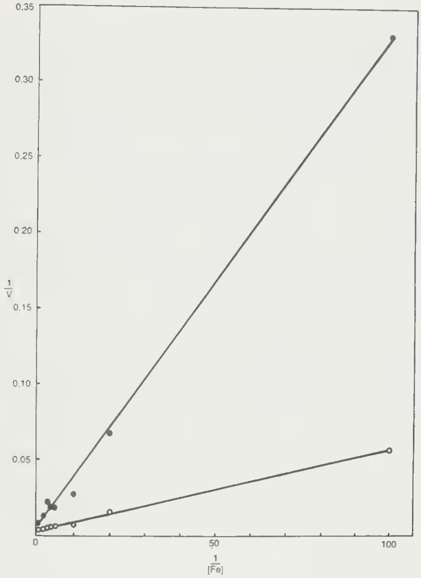

FIG. 3.
Kinetic analysis of results in Figure 2—low concentration range (0,01–1 mM Fe). ●—● leaf discs; ○—○ root tips.

The possibility exists that Fe in the high concentration range is precipitated in the tissue, which might explain the exponential rise of the curves. Tissue damage due to the high Fe concentrations with accompanying passive inward movement, appears unlikely, since in that case one would expect the desorption treatment with the chelate to remove that part of the absorbed Fe, which apparently did not occur (compare Figures 1 and 2).

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REFERENCES

ALLEN, J. E. 1970. *The preparation of agricultural samples for analysis by atomic absorption spectroscopy*. Springvale, Australia: Varian Techtron.

EPSTEIN, E., 1966. Dual pattern of ion absorption by plant cells and by plants. *Nature* **212:** 1324–1327.

EPSTEIN, E., 1972. *Mineral nutrition of plants: principles and perspectives*. New York: John Wiley and Sons, Inc.

EPSTEIN, E., 1976. Kinetics of ion transport and the carrier concept. In: Lüttge, U. and Pitman, M. G. (eds.), *Encyclopedia of Pl. Physiol.*, New Series, Volume 2 Part B. pp. 70–94 Berlin, Heidelberg, New York: Springer-Verlag.

EPSTEIN, E., RAINS, D. W. and ELZAM, O. E., 1963. Resolution of dual mechanisms of potassium absorption by barley roots. *Proc. natn. Acad. Sci. U.S.A.* **49:** 684–692.

HOAGLAND, D. R. and ARNON, D. I., 1938. The water-culture method for growing plants without soil. *Calif. Agric. Exp. Sta. Cir.* **347.**

KANNAN, S., 1971. Kinetics of iron absorption by excised rice roots. *Planta* **96:** 262–270.

TORII, K. and LATIES, G. G., 1966. Dual mechanisms of ion uptake in relation to vacuolation in corn roots. *Pl. Physiol., Cutz Town* **41:** 863–870.